

Amendments to th Specification:

Please amend paragraph 4 of the specification on page 1 to read as follows:

[0004] The green fluorescent protein (~~GFIP~~) (GFP) of the jellyfish *Aequorea victoria* is a remarkable protein with strong visible absorbance and fluorescence from a p-hydroxybenzylideneimidazolone chromophore, which is generated by cyclization and oxidation of the protein's own Ser-Tyr-Gly sequence at positions 65 to 67. A cDNA sequence {[SEQ ID NO:1]} for one isotype of GFP has been reported [Prasher, D. C. et al., Gene 111, 229-233 (1992)]; cloning of this cDNA has enabled GFP expression in different organisms. The finding that the expressed protein becomes fluorescent in cells from a wide variety of organisms [Chalfie, M. et al., Science 263, 802-805 (1994)] makes GFP a powerful new tool in molecular and cell biology and indicates that the oxidative cyclization must be either spontaneous or dependent only on ubiquitous enzymes and reactants.

Please amend paragraph 53 of the specification on page 18 to read as follows:

[0053] Random mutagenesis of the gfp GFP cDNA was done by increasing the error rate of the PCR with 0.1 mM MnCl₂ and unbalanced nucleotide concentrations. The GFP mutants S65T, Y66H and Y66W had been cloned into the BamHI site of the expression vector pRSETB (Invitrogen), which includes a T7 promoter and a polyhistidine tag. The GFP coding region (shown in bold) was flanked by the following 5' and 3' sequences: 5'-G GAT CCC CCC GCT GAA TTC ATG . . . AAA TAA TAA GGA TCC-3' (where 5'-G GAT CCC CCC GCT GAA TTC ATG-3' is SEQ ID NO: 3 and 5'-AAA TAA TAA GGA TCC-3' is SEQ ID NO: 4). The 5' primer for the mutagenic PCR was the T7 primer matching the vector sequence; the 3' primer was 5'-GGT AAG CTT TTA TTT GTA TAG TTC ATC CAT GCC-3', (SEQ ID NO: 5) specific for the 3' end of GFP, creating a HindIII restriction site next to the stop codon. Amplification was over 25 cycles (1 min at 94° C., 1 min 52° C., 1 min 72° C.) using the AmpliTaq polymerase from Perkin Elmer. Four separate reactions were run in which the concentration of a different nucleotide was lowered from 200 μM to 50 μM. The PCR products were

combined, digested with BamHI and HindIII and ligated to the pRSETB cut with BamHI and HindIII. The ligation mixture was dialyzed against water, dried and subsequently transformed into the bacterial strain BL21(DE3) by electroporation (50 µl electrocompetent cells in 0.1 cm cuvettes, 1900 V, 200 ohm, 25 µF). Colonies on agar were visually screened for brightness as previously described herein. The selected clones were sequenced with the Sequenase version 2.0 kit from United States Biochemical.

Amendments to the Sequence Listing:

Please amend the Sequence Listing to include the following three sequences:

SEQ ID NO: 3

5'-G GAT CCC CCC GCT GAA TTC ATG-3'

SEQ ID NO: 4

5'-AAA TAA TAA GGA TCC-3'

SEQ ID NO: 5

5'-GGT AAG CTT TTA TTT GTA TAG TTC ATC CAT GCC-3'